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A PRELIMINARY STUDY OF THE HYDROGEN ION CONCENTRATION IN WESTERN YELLOW PINE IN RELATION TO BARK BEETLE ATTACK

by

HOUGHTON DURBROW
Bureau of Entomology

University of California
Berkeley, California
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A PRELIMINARY STUDY OF THE HYDROGEN ION CONCENTRATION IN WESTERN YELLOW PINE IN RELATION TO BARK BEETLE ATTACK

Introduction

The attraction studies conducted by Person[1] and Jeffrey[2] in 1929 with Dendroctonus brevicomis Lec. seemed to indicate a possible relationship between the attractiveness of the tree to insects and the hydrogen ion concentration of the inner bark. The work reported here was undertaken to determine whether such a relationship existed, and if so, to what extent.

The studies were conducted during the 1930 field season in the Bureau of Entomology field laboratories, located at the Buck Creek Ranger Station and near Hackamore, on the Modoc National Forest, California. The timber type in this area is pure yellow pine, in a mixed or all-age stand.

The author is sincerely grateful to Mr. H.L. Person for his help in planning the work and editing the report, and to Mr. R.N. Jeffrey, under whose immediate supervision the work was carried out.

Methods

Because of the nature of the problem and the location of the field laboratories we were limited to the use of portable apparatus. Through the courtesy of the Division of Entomology of the University of California we obtained the use of a Welch quinhydrone apparatus, with which we compared the results of the colorimetric method. In the preliminary experiments the quinhydrone apparatus was so difficult to handle and gave such inconsistent and widely-varying results that it was abandoned early in the work, and all subsequent determinations were made by using the standard color indicators.

The means by which a solution was to be made from the inner bark presented considerable difficulty. As no work of this nature using the inner bark of pine trees has been done, it was necessary to develop our own methods for this study. Several different ones were tried and rejected before one was finally developed and adopted as standard for the remainder of the season. Those tried were:

1. Soaking strips of the inner bark for various periods of time in distilled water and reading the pH of the infusion;

2. Grinding the bark in a meat chopper and soaking the pulp in distilled water, various times and degrees of fineness being tried;

3. Grinding with mortar and pestle and soaking the pulp in distilled water;

4. Direct staining of a piece of the inner bark with the indicator solutions.

Late in July a small bronze press resembling an ordinary grease gun was obtained and used for the remainder of the season. Using the press for extracting the cell sap, a method was developed which proved to be more satisfactory than any of the others. In detail, the method finally adopted is as follows:

1. Sampling. The dead outer bark was chipped away from the tree with an axe until it was from one-quarter to one-half inch in thickness and then, using the axe as a chisel, the remainder of the bark was removed. Great care was taken not to cut deeply into the soft inner bark or bruise it with heavy blows. Best results were obtained by holding the axe by the head and pushing upward, rather than by using a chopping stroke. After the inner bark had been completely exposed a strip approximately 4" by 20" (larger or smaller, depending upon the thickness of the bark) was outlined lengthwise of the tree by cutting through it with a pocket knife. After this the inner bark was pried away from the tree by inserting a dull knifeblade under one corner and working it carefully across the top of the strip. After removing the sample it was placed in a screw-top jar and taken to the laboratory, where the determinations were run. If the sampling is done in the spring or early summer, when the tree is making its best growth, the bark can usually be pulled free of the tree. In late summer and fall, however, it is usually necessary to remove the bark by cutting it free from the tree, cutting across the strip through the cambium cells and pulling at the same time.

Two experiments were run in an effort to determine the deterioration which the sample might undergo between the time it was removed from the tree and the time when it was worked. The results of these experiments indicate that no appreciable change takes place over a period of several hours.

The place on the tree from which the sample is taken has a definite effect upon the value obtained. Results of the four experiments to determine the amount of change with position are given in Table I. Because of this variation all samples were removed from the west side of the tree, the center of the sample being at breast height, or 4½ feet above the ground.

TABLE I

Values of pH Determinations from Eight Positions on the Tree

Tree No.	Crown Class	pH							
		Stump	Breast Height				Middle of Bole	Base of Crown	Center of Crown
		N.Side	N.Side	E.Side	S.Side	W.Side	N.Side	N.Side	N.Side
50	4	4.40	4.50	4.70	4.75	4.70	4.70	4.70	4.75
45	-	4.40	4.50	4.60	4.60	4.60	4.55	4.60	4.60
46	1	4.40	4.45	4.50	4.45	4.45	4.55	4.90	4.80
48	1	4.60	5.00	4.90	4.80	4.90	5.00	4.80	4.80

2. Grinding. Grinding the sample was done as soon after sampling as possible. In no case was a sample ever held more than two or three hours before it was worked. The grinding was done in a #0 Russwin mill, or meat chopper. The strip of bark was first run through the mill, using the coarse cutter, and then twice, using the nut-butter cutter, the resulting sample being mixed thoroughly after each grinding. After the final grinding the bark had the consistency of a fairly stiff dough or of wet cornmeal, depending upon the moisture in the sample. An effort was made to have at least twice as much ground bark as was required to fill the press, which held about 35 grams.

The grinder had a tendency to cause a grayish color in the ground material, probably due to the formation of iron tannate; and for this reason an effort was made to determine the change in the pH that might be due to this cause. No sample could be obtained that was absolutely free from tannate, but dark and light portions of the same sample showed no differences. However, if a grinder made of brass could be obtained, it would undoubtedly be more satisfactory.

3. Pressing, or extracting the cell sap. The dimensions and type of the press used are shown in Fig. 1. This was made on order at one of the local machine shops. The tendency of the ground pulp to pack tight in the bottom of the press prevented all but a small fraction of the moisture from coming through. This could probably be overcome by experimenting with various sizes and types of screens or combinations of these. The quantity of juice expressed was from 3 to 15 cc., depending upon the moisture in the sample.

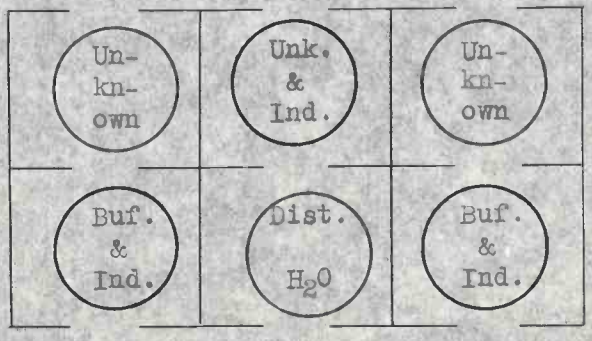
Both the grinder and the press were thoroughly cleaned before another sample was worked, great care being taken to remove all traces of the iron tannate and the old sample.

4. Determinations. Because of the limited time available for the work, and the possibility of having to use small quantities of solution for making determinations, it was decided to make up a set of color standards adapted to 5 cc. samples rather than to use a set of commercial stamdards. The buffer solutions were made according to Clark and Lubs, as found in Clark's "Determination of Hydrogen Ions". After the buffer mixtures had been made they were checked against the quinhydrone apparatus and found to agree within 0.02 pH with the calculated values except pH 6.0, which was 6.03. Sufficient test tubes $\frac{1}{2}$x3 inches were selected from a large number, care being taken to get a set that was uniform as to size, color and uniformity of glass. These were drawn to a fine point in a gas flame, and the tip broken so as to leave a neck on the vial about $1\frac{1}{2}$ inches long and about 1/8 inch in diameter. After the vials had cooled they were soaked several hours and then drained and dried. The vials were filled with a fine-tipped funnel made by drawing an ordinary test tube to a small point. Five drops of the 0.04% indicator solution were placed in the funnel and then washed into the ampule with 5 cc. of the required buffer mixture. To avoid contamination by several transfers, the buffer solution was measured by filling the ampule to a mark that had been previously made. A standard pattern block comparator of a size to take these tubes was also made.

The juice as it came from the press contained a large amount of starch and other suspended matter, and for this reason it was necessary to dilute 10:1 with distilled water. Dilutions as high as 50:1 were tried and found to give the same values as 10:1 dilutions; and it was therefore assumed that a 10:1 dilution would be legitimate practice. A titration curve of this 10:1 solution is shown in Fig. 2, which shows a flattening of the curve at pH 4.4, the normal value for this sample.

In practice three of the test tubes containing 5 cc. portions of the solution of bark juice were placed in the back (or lighted side) of the comparator, and to the center one 5 drops of the indicator solution were added. In the hole in front of this tube a test tube of distilled water was placed, and on either side of this one of any adjacent pair of color standards for the particular indicator being used. The light coming from behind thus passes through the same quantities of colored and uncolored materials:

(Light)



(Eye)

While it is contrary to usual practice to place the "unknown" solution behind the color standards, it was found necessary in this case because of the Tyndall effect, or the dispersion of the beam of light as it passes through the colloidal suspension.

The color standards were changed until the one was found that most closely matched the color produced in the center hole. With care and a little experience determinations to 0.05 pH are quite possible.

Results Obtained

Several circumstances, such as the short field season, the long time required to work out a satisfactory method and the late date at which the press was procured, conspired to limit the amount of data that could be taken; consequently it is not possible to draw many definite conclusions. However, there are a sufficient number of determinations to show that as a practical field method of selecting trees susceptible to insect attack the pH of the inner bark has little or no value. The principal contribution from the work is the development of a field method for determining the pH of material of this nature, and further support for the selection theory as developed by Person and Jeffrey. Trees selected as highly susceptible have a pH slightly higher or lower than normal healthy trees. This gives further evidence of some chemical condition in attractive trees that is associated with abnormal enzyme activity.

1. Green Trees. The pH of 32 green trees was determined, these values being tabulated in the summary table. The trees were distributed through the various crown classes (Dunning's classification) as follows:

Class 1	- - 12	trees
" 3	- - - 4	"
" 4	- - 2	"
" 5	- - -14	"

In Fig. 3 are shown the extreme values obtained and the averages for the group as a whole and for the various crown classes. More Class 1 and Class 5 trees were selected, as these two classes represent the extremes in attractiveness to insect attack (3), and any relationship between attractiveness and pH should therefore be more apparent. In practice, however, this was not found to be the case. Class 1 trees had the same spread of values as did the group as a whole, and the same average value. Class 5 trees also had nearly the same spread but tended to average slightly lower. This would indicate that this group gave some slight indication of attractiveness by tending to be more acid, but the relationship is not sufficiently marked to enable one to draw any conclusions. In Classes 3 and 4 there were too few trees on which to base any definite statements.

An attempt was made to correlate growth rate with the pH determinations. The mean annual growth for the past five years was determined, and the pH of the inner bark plotted over the growth rate for the corresponding tree (Fig. 4). An examination of these data will show that no correlation exists, and that the line of average pH fits the data as well as any other line that might be drawn. The same thing was done using only the Class 5 trees, with similar results.

2. Attacked Trees.. Only one sample was obtained from a standing tree which was being attacked by D.b. The egg galleries in this sample were over ten inches in length and much crossed. The sample had a pH of 4.85. Two other samples from the unattacked portions of similar trees were pH 4.8 and 5.0.

Samples from two trap logs were also obtained, the egg galleries in these being from one to three inches in length. These samples were both 4.5 pH. Here again the data are too few to be conclusive. In the case of the standing tree the galleries were much longer than those in the trap logs, and the higher pH might therefore be due to changes brought about by the attack. If this is true, then the changes must be rather widespread throughout the tree during or shortly after attack, as shown by the values obtained from the uninfested portions. This would indicate that although pH in itself cannot be used as an index of attractiveness, an abnormal pH is probably associated with changes which do affect the attractiveness of the tree.

Samples taken from the inner bark of trees in which the brood had gone over into the outer bark had pH values of 5.3, 5.5 and 5.6, while the outer bark of similar trees was found to be between 3.2 and 3.8. Two conclusions are possible here: (1) that here is further evidence that the pH of the inner bark increases with attack and with the development of the brood; and (2) that the larvae leave the inner bark after it becomes too alkaline, seeking the more acid outer bark.

For purposes of comparison these values are tabulated in Table II:

TABLE II
pH Values of Attacked Samples and Stage of Attack

Tree No.	Portion	pH	Stage of Attack
46	Inner	4.5	Trap log; galleries about 3" long
49	"	4.5	Trap log; galleries 1-3 inches long
22	"	4.85	Egg galleries over 10" long
24	"	4.8	Attacked tree; no attack on sample
23	"	4.8	" "
31	Inner	5.5	Mature larvae in outer bark
	Outer	3.8	
32	Inner	5.3	Mature larvae in outer bark
	Outer	3.4	
30	Inner	5.6	Larvae in outer bark

3. Top-Killed Trees. Determinations upon three top-killed trees (Ips) showed these trees to be uniformly high as regards pH, the values being 5.0, 5.1 and 5.2. This is apparently a contradiction of other data, as the slower-growing, less vigorous trees tended to have a lower pH. However, this high value might be due to the type of injury, insect attack having already been shown to raise the pH throughout the tree. Sufficient data have not been gathered to show more than that any abnormal pH might be associated with attractiveness, whether it were high or low.

4. Mechanically-Injured Trees. Determinations were run upon eight trees that had been mechanically injured in connection with Jeffrey's sugar determinations. The injuries were made in May, 1930, and consisted of girdling, defoliation and root-pruning. By the middle of August, approximately three months later, no apparent difference could be noted between these and the normal green trees.

Two trees that were sampled extensively in 1929 were also included in the study and found to have pH values below those of the normal trees. The pH of these trees was 4.4 and 4.5. The indication here is that the effect produced by mechanical injury is not apparent until the second season, when it is manifested by an increase in acidity. This might account for the attractiveness of injured trees in the second season, as the pH of these two trees is practically that of the attacked samples from trap logs.

The extreme and average values for all injured and attacked samples are shown graphically in Fig. 6, as well as those for the entire 32 green trees.

5. Trap Logs. Three trees were felled for trap logs, with the intention of following any changes in the pH that might occur by reading one sample every seven days. However, the season closed sooner than expected, and readings were obtained only over a period of two weeks on one tree and to the end of the first week on the second tree. These trees did show a movement toward a greater acidity than normal. A total change of 0.4 pH was observed after a period of two weeks, but practically no change during the first seven days. In Fig. 5 these data are shown graphically.

Conclusions

From the results of the work this past season it is concluded that the colorimetric methods are better adapted for taking pH under field conditions than any of the other known methods. They are less costly, results sufficiently accurate are easily obtainable, and the necessary equipment can easily be carried in a car without danger of damaging costly and delicate instruments.

Apparently pH is not a satisfactory index of the health or attractiveness of trees. Differences between the healthiest individuals of the same class or group are nearly as large as those between the weakest and healthiest trees of different tree classes. We may assume that so far as practical use in selecting attractive trees is concerned, pH is of little positive value. Recognizing this, however, the study is of value in support of the selection theory, which assumes a respiratory fermentation with abnormal enzyme activity as a basis for the greater attractiveness of certain trees. This condition might be indicated by an H-ion concentration either above or below the normal.

In spite of the small amount of data the results indicated:

1. A pH below normal at the time of attack on trap logs;

2. A tendency for injured trees to approach this attractive pH during the season following the injury;

3. An increase in pH shortly following attack and increasing during the development of the brood;

4. No correlation between rate of growth and the pH of the inner bark; and

5. A change in trap logs away from the normal and toward the acid side of the range.

SUMMARY TABLE

Tree No.	D.B.H. Inches	Height Feet	Crown Class	* Crown Type	Mean Annual Growth mm.	pH	Remarks
64	22	90	1	D	1.25	4.6	Normal green tree
27	28	125	1	D	1.36	5.0	do
48	22	75	1	D	1.70	4.9	See Table I
46	22	110	1	X	1.82	4.45	do
63	20	100	1	D	1.92	4.7	Normal green tree
45	24	100	1	D	2.04	4.6	See Table I
70	16	85	1	D	2.15	4.65	Normal green tree
71	18	80	1	D	2.80	4.7	do
62	18	75	1	D	2.90	4.8	do
61	16	75	1	D	2.94	4.65	do
59	20	70	1	D	3.22	4.7	Normal green tree
60	22	100	1	D	5.04	4.6	do
29	28	115	3	C	1.69	4.6	do
26	26	115	3	X	2.30	5.0	do
33	--	--	3	D	1.70	4.6	do
14	--	--	3	D	1.72	4.55	Normal green tree
65	16	80	4	C	0.44	4.6	do
50	24	90	4	I	1.32	4.7	See Table I
68	20	75	5	C	0.11	4.6	Normal green tree
66	22	100	5	C	0.18	4.6	do
58	26	100	5	C	0.27	4.8	Normal green tree
56	30	115	5	D	0.28	5.0	do
54	54	130	5	D	0.41	4.7	do
67	18	105	5	C	0.45	4.7	do
57	40	150	5	D	0.53	4.6	do
69	26	70	5	C	0.62	4.85	Normal green tree
52	60	130	5	D	0.63	4.55	do
52	46	120	5	D	0.82	4.55	do
53	36	110	5	X	0.87	4.55	do
18	--	--	5	D	0.88	4.55	do
13	--	---	5	D	1.02	4.6	Normal green tree
55	34	---	5	D	1.49	4.7	do
49	24	80	4	S	0.18	4.8	Slash tree #21; sampled 25 d. after felling
49	--	--	-	-	----	4.5	Attacked sample; do
47	24	90	1	D	2.12	5.15	Slash tree #21; do
28	48	120	5	D	0.98	5.1	Ips top-killed tree; 25% defoliation
21	34	100	5	D	0.44	5.0	do 25% "
20	34	110	5	D	----	5.2	do 90% "
22	22	90	4	C	0.53	4.85	Attacked sample; egg galleries of D.b. about 10"
24	34	110	5	D	----	4.8	" tree; sample from unattacked area
23	24	100	4	C	0.73	5.0	Attacked tree; sample from unattacked area
30	30	120	5	D	0.36	5.6	" sample; mature larvae in outer bark
31	50	120	5	D	0.35	5.5	" " brood emerged
32	46	120	5	D	0.34	5.3	" " mature larvae in outer bark
31	--	---	-	-	----	3.8	pH of outer bark
32	--	---	-	-	----	3.4	pH of outer bark
32a	--	---	-	-	----	3.2	pH of outer bark from trap log
42	--	---	-	-	6.01	4.4	Tree extensively sampled in 1929
43	--	---	-	-	7.36	4.45	do
44	20	75	1	D	7.63	4.7	Tree deeply blazed in 1929, 2x2 feet
34	20	80	1	C	0.98	4.55	Check tree, uninjured except for sampling
35	22	90	3	C	1.80	4.60	do
36	22	85	3	C	1.56	4.60	Cambium girdle; pH above girdle
36	--	--	-	-	----	4.50	do pH below "
38	22	95	1	C	1.00	4.65	Root pruned; 50% of roots cut through
37	22	95	1	D	1.57	4.80	Deep girdle; pH above girdle
37	--	--	-	-	----	4.60	do pH below "
39	20	80	1	I	0.95	4.7	75% defoliation by pruning
40	32	90	1	C	2.73	4.8	Ground fire, severe scorch of base
41	22	60	1	X	3.01	4.75	66% defoliation by fire

(Mechanical injury)

*Crown Types: D, Dominant; C, Codominant; I, intermediate; S, Suppressed; X, Isolated
Mean annual growth calculated over period 1925-29 inclusive

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(2) JEFFREY, R.N. The Concentration of Certain Sugars in the Bark of Western Yellow Pine, as Related to Western Pine Beetle Attraction; March 1930

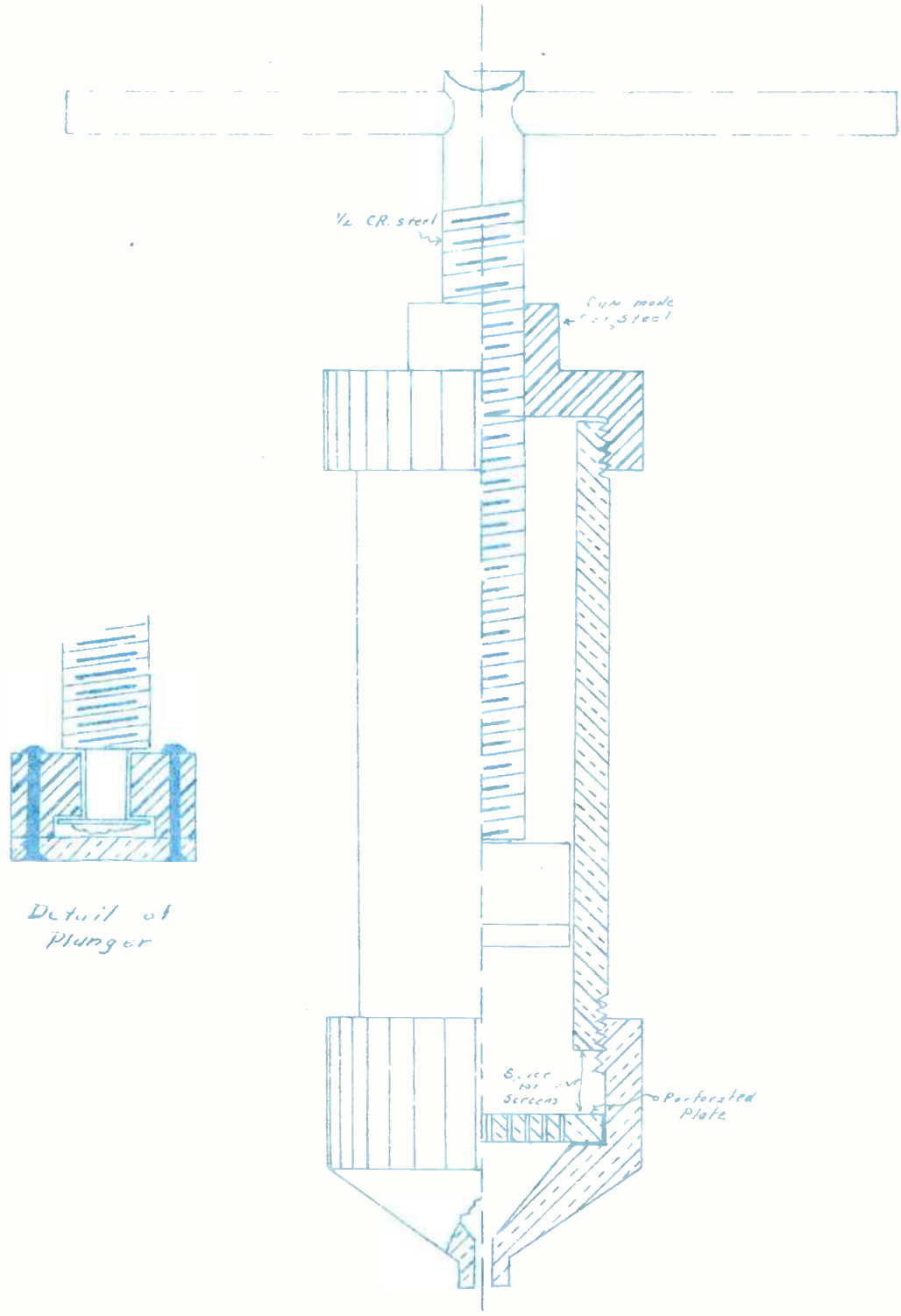
(3) PERSON, H.L. Sample Plot Studies of Tree Selection by the Western Pine Beetle; unpublished report

Fig. 1. Press used in extracting the cell sap. The drawing is full size and no dimensions are given, as these can be varied to fit the stock available in the machine shop. All parts are of brass except the working members, which are of cold-rolled steel.

Fig. 1

Burk Press

Scale 1" = 1"



Detail of Plunger

Figure 2

Titration Curve of the 10:1

Dilution of Bark Juice

Note the flattening at pH 4.4, which shows the resistance (or "buffer effect") of the solution to changes in hydrogen ion concentration with the addition of relatively concentrated acid and base.

Fig. 2

Titration Curve

of

10:1 Dilution of Bark Juice

(25 c.c.)

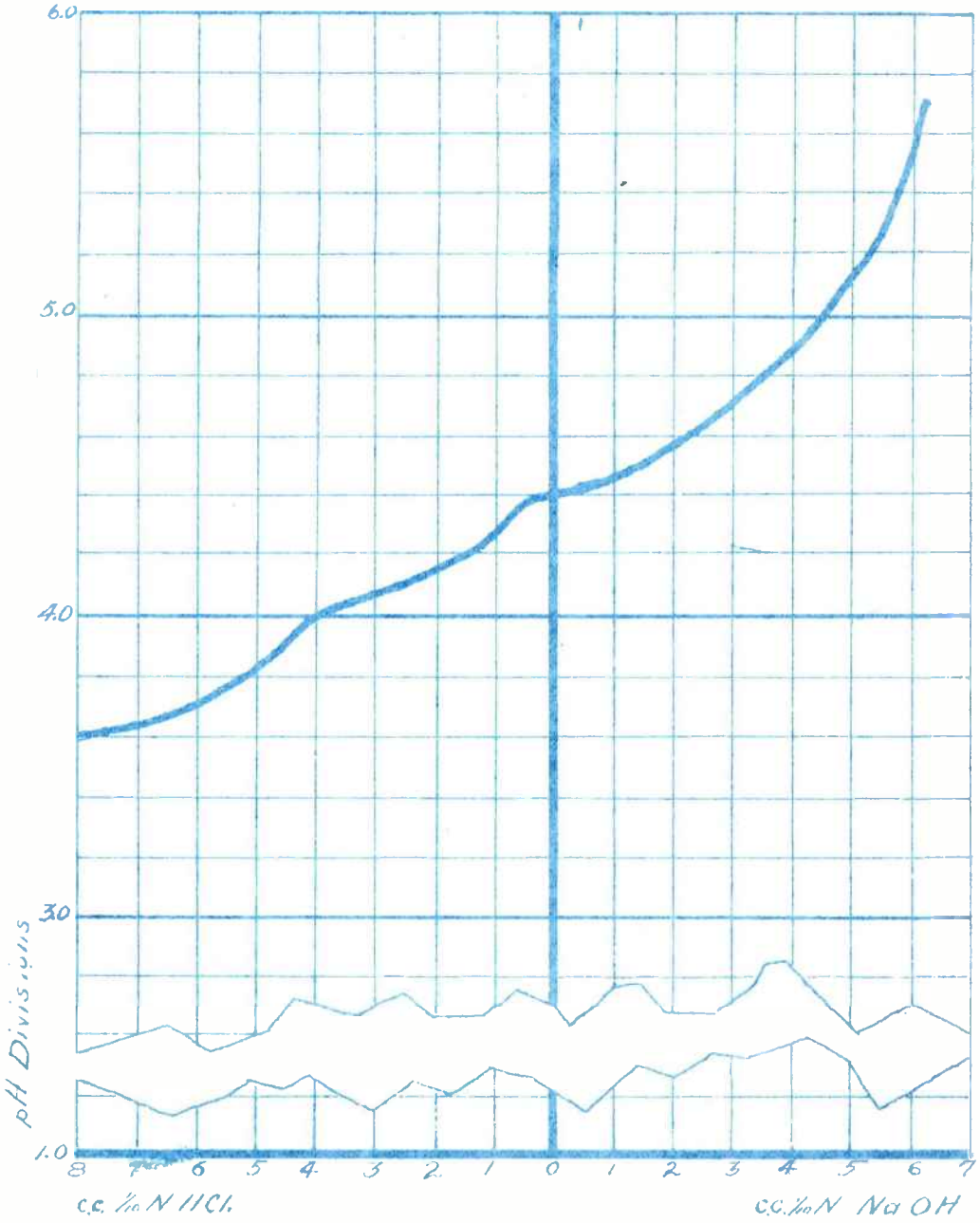


Figure 3

Extreme and Average pH Values
of 32 Green Trees,
Grouped According to Crown Classes

Extreme values are indicated as the top and bottom of the vertical bars, and the average for the group as a short horizontal bar. Note that each class has nearly the same spread as that of the entire 32 trees(except Class 4, with only two trees) and that Class 5 shows a slight tendency toward a lower (more acid) pH.

Fig. 3.

Extreme and Average pH Value

32 Green Trees

According to Crown Classes

Spread shown by vertical bar diagrams

Average value by horizontal bar

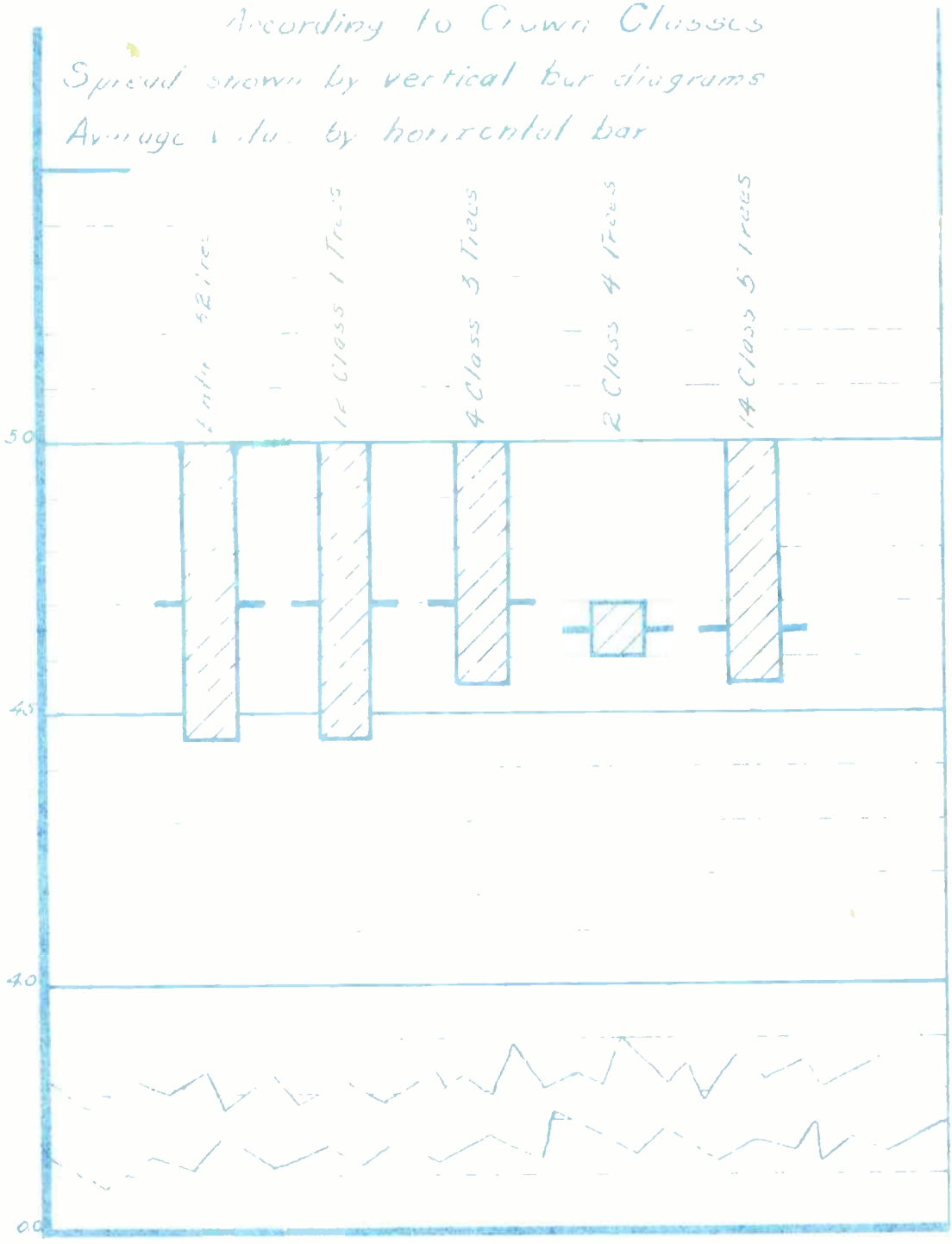


Figure 4

Relationship Between Mean Annual Growth and the pH of the Inner Bark

Practically no correlation can be drawn from these data, so great is the dispersion. The line of average pH seems to fit the data as well as any other line that might be drawn.

Figure 5

Changes Occurring in Trap Logs

These data have been referred to a common base, and plotted as change in pH.

Fig. 4

Relationship Between
Mean Annual Growth and
pH of Inner Bark

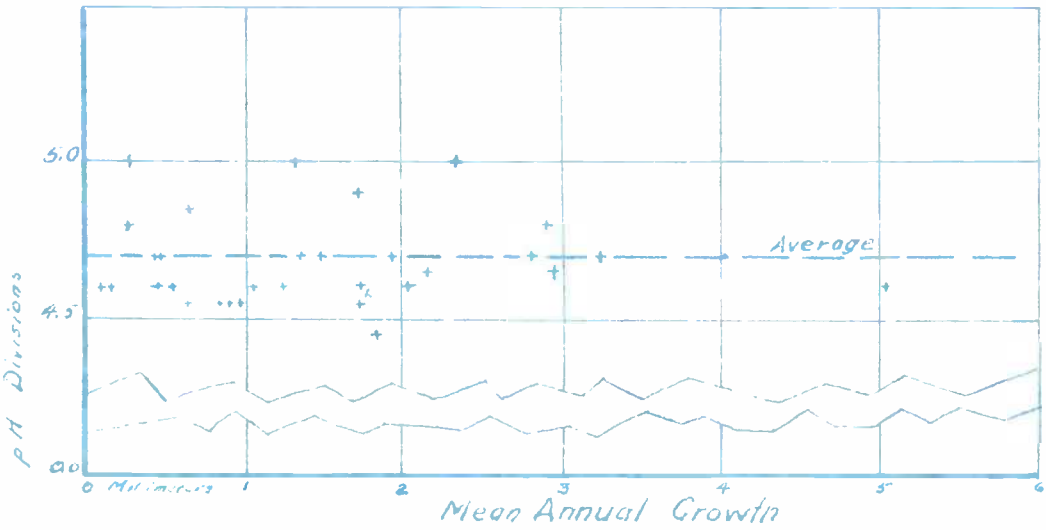


Fig. 5

Changes in pH
Occuring in Trap-logs

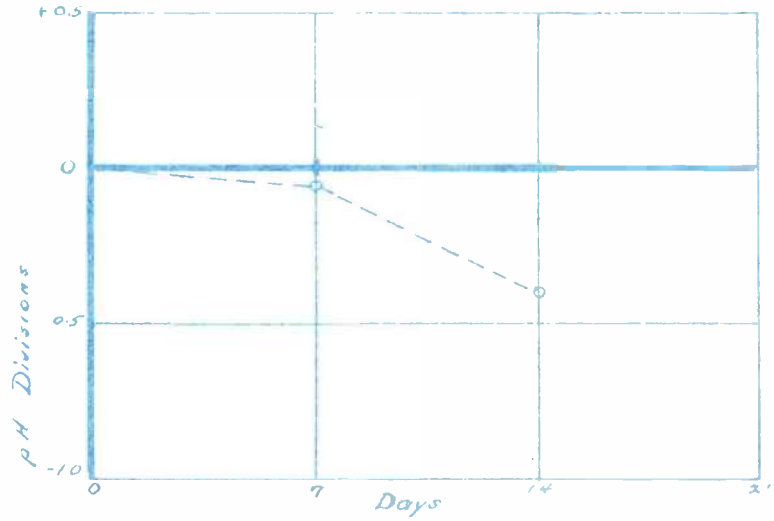


Figure 6

Extreme and Average pH Values from

Attacked and Injured Trees

Shows graphically the relative pH values of attacked and injured trees as compared with the 32 green trees. Note that nearly all the values fall outside the range of the green trees.

Fig. 6

Extreme and Average pH Values

of Samples from

Injured and Attacked Trees